In re Appln. of Sogabe et al.
Application No. 09/940,941

CLAIM AMENDMENTS

IN THE CLAIMS:

1.-32. (cancelled)

33. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by ~~mutation of~~ mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point of 4.5.

34. (canceled)

35. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by ~~mutation of~~ mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

36. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by ~~mutation of~~ mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b)

a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

37. (currently amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by ~~mutation of~~ mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

38. (currently amended) A method for producing the creatine amidinohydrolase of claim ~~24~~ 33, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

39. (currently amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim ~~24~~ 33, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

40. (currently amended) A method for determining creatine in a sample, which comprises measuring ~~an~~ absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.

41. (currently amended) A reagent for determination of creatinine in a sample, comprising a creatinine ~~amidinohydrolase~~ amidohydrolase, the creatine amidinohydrolase of claim ~~24~~ 33, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

42. (currently amended) A method for determining creatinine in a sample, which comprises measuring ~~an~~ absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.

43. (currently amended) A method for producing the creatine amidinohydrolase of claim ~~25~~ 35, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

44. (currently amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim ~~25~~ 35, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

45. (currently amended) A method for determining creatine in a sample, which comprises measuring ~~an~~ absorbance of a pigment produced by the reaction of the reagent of claim 44 with the sample.

46. (currently amended) A reagent for determination of creatinine in a sample, comprising a creatinine ~~amidinohydrolase~~ amidohydrolase, the creatine amidinohydrolase of claim ~~25~~ 35, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

47. (currently amended) A method for determining creatinine in a sample, which comprises measuring ~~an~~ absorbance of a pigment produced by the reaction of the reagent of claim 46 with the sample.

48. (currently amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5 - 10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

49. (currently amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

50. (currently amended) The creatine amidinohydrolase of claim 49, which is obtained from ~~*Escherchia*~~ *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375).

51. (currently amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

52. (currently amended) The creatine amidinohydrolase of claim 51, which is obtained from ~~*Escherchia*~~ *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374).

53. (currently amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H_2O → sarcosine + urea

Optimum temperature: about 40-50° C (at ~~a~~ pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5

54. (currently amended) The creatine amidinohydrolase of claim 53, which is obtained from ~~*Escherchia*~~ *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

55. (previously presented) A method for producing the creatine amidinohydrolase of claim 48, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

56. (currently amended) The method of claim 55, wherein said microorganism is selected from the group consisting of ~~*Escherchia*~~ *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374), ~~*Escherchia*~~ *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375), ~~*Escherchia*~~ *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

57. (previously presented) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

58. (previously presented) The reagent of claim 57, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore, and a buffer.

59. (previously presented) The reagent of claim 58, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.

60. (previously presented) The reagent of claim 58, in which the chromophore comprises a hydrogen receptor and a coupler.

61. (previously presented) The reagent of claim 60, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

62. (previously presented) The reagent of claim 60, in which the coupler is an aniline derivative or a phenol derivative.

63. (currently amended) A method for determining creatine in a sample, which comprises measuring ~~the~~ absorbance of the pigment produced by the reaction of the reagent of claim ~~49~~ 57 with the sample.

64. (previously presented) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

65. (previously presented) The reagent of claim 64, in which the composition for the detection of hydrogen peroxide comprises and enzyme having a peroxidase activity, a chromophore, and a buffer.

66. (previously presented) The reagent of claim 65, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.

67. (previously presented) The reagent of claim 65, in which the chromophore comprises a hydrogen receptor and a coupler.

68. (previously presented) The reagent of claim 67, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

69. (previously presented) The reagent of claim 67, in which the coupler is an aniline derivative or a phenol derivative.

70. (currently amended) A method for determining creatinine in a sample, which comprises measuring ~~the~~ absorbance of the pigment produced by the reaction of the reagent of claim 64 with the sample.

71. (new) A method of preparing a creatine amidinohydrolase comprising:

(i) mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 to provide mutant nucleic acid sequences,

(ii) determining Km values of proteins encoded by the mutant nucleic acid sequences in a coupling assay using a sarcosine oxidase and a peroxidase,

(iii) selecting and isolating a desired mutant nucleic acid sequence that encodes a creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM,

(iv) expressing the desired mutant nucleic acid sequence in a host to produce creatine amidinohydrolase, and

(v) harvesting the produced creatine amidinohydrolase.

72. (new) The method of claim 71, wherein the creatine amidinohydrolase has a molecular weight of about 43,000 (SDS-PAGE).

73. (new) The method of claim 72, wherein the creatine amidinohydrolase has an isoelectic point of about 4.5.

74. (new) The method of claim 73, wherein the creatine amidinohydrolase has an optimum temperature of about 40-50 °C (at pH of about 7.5).

75. (new) The method of claim 74, wherein the creatine amidinohydrolase has an optimum pH of about 8.0-9.0 (at a temperature of about 37 °C).